

# Nesting behavior and ecological preferences of five Diphaglossinae species (Hymenoptera, Apoidea, Colletidae) from Argentina and Chile

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Academic editor: Jack Neff | Received 6 March 2013 | Accepted 17 July 2013 | Published 1 August 2013

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**Citation:** Sarzetti LC, Genise FF, Sánchez VM, Farina JL, Molina AM (2013) Nesting behavior and ecological preferences of five Diphaglossinae species (Hymenoptera, Apoidea, Colletidae) from Argentina and Chile. Journal of Hymenoptera Research 33: 63–82. doi: [10.3897/JHR.33.5061](https://doi.org/10.3897/JHR.33.5061)

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## Abstract

The nests of *Cadeguala albopilosa* (Spinola, 1851), *Diphaglossa gayi* Spinola, 1851, *Ptiloglossa tarsata* (Friese, 1900), *Ptiloglossa matutina* (Schrottky, 1904) and *Zikanapis tucumana* (Moure, 1945) (Colletidae, Diphaglossinae) from Argentina and Chile are described herein. They show similar features to those of other Diphaglossinae: they consist of a main tunnel, cells disposed radially, isolated or in pairs, and connected to the main tunnel by laterals ones. Main tunnels are mostly vertical in species nesting in soil surface but horizontal to inclined in *D. gayi*, which nests in banks. Cells are vertical with curved necks. The cells of *C. albopilosa* show less curved necks (less than 90°), whereas in the remaining four species the cell neck is highly curved (90° or more). Cells of *P. tarsata* have a spiral earthen closure and a wad cotton-like material, whereas in *P. matutina* only had the last one. In the remaining studied species any type of closure were found. Cocoons of *C. albopilosa* and *P. tarsata* are coriaceous showing a closure composed of three disks. *Zikanapis tucumana* and possibly *P. matutina* showed dim-light foraging. The remaining species are diurnal. The climate in their nesting sites is highly diverse, ranging from 8°C to 20°C in mean annual temperature, and from 250 mm to 3000 mm in mean annual precipitation. Only *C. albopilosa* and, to a lesser extent, *Z. tucumana* nested gregariously. *Zikanapis tucumana* and *P. tarsata* were observed visiting flowers of *Solanum*.



## Keywords

*Cadeguala albopilosa*, *Diphaglossa gayi*, *Ptiloglossa tarsata*, *Ptiloglossa matutina*, *Zikanapis tucumana*, Caupolicanini, Diphaglossini, nest architecture, ecological preferences

## Introduction

The Diphaglossinae include the largest and most robust Colletidae. They are known for being mostly crepuscular to nocturnal bees that are found in habitats ranging from deserts or near deserts to humid tropical rain forests (Rozen 1984, Almeida et al. 2012). Michener (2007) included nine genera in this subfamily distributed in three tribes, which are restricted to the New World. In contrast, Urban et al. (2012) considered this subfamily as a tribe divided in the subtribes Caupolicanina, Diphaglossina, and Dissoglottina, which include eleven genera. The genus *Zikanapis* is distributed in warm temperate areas of the continent (Compagnucci 2006) and includes five species from Argentina (Urban and Moure 2001, Michener et al. 2003, Compagnucci 2006, Urban et al. 2012). The genus *Ptiloglossa* includes 30 or more species ranging from Argentina to Texas and Arizona in USA (Michener 2007). The genus *Cadeguala* contains two species distributed from the Coquimbo region in northern Chile and Bolivia to Valdivia in southern Chile, and Río Negro province, Argentina (Michener 2007, Montalva et al. 2011). Finally, *Diphaglossa*, with only one species endemic to Chile, occurs from 30° to 50° of latitude in the continent and in the Chiloé island (Michener 2007, Montalva and Ruz 2010, Montalva et al. 2011).

The behavior and nest architecture of all Diphaglossinae is rather homogeneous and contrasts markedly with that of other colletid subfamilies (Janvier 1955, Roberts 1971, Rozen 1984). Typically, nesting behavior consists of the excavation of a vertical main tunnel by a single female and horizontal laterals ending in a large, vertically oriented cell with fluid provisions. Two of the most interesting features of diphaglossine biology are the construction of curved cells and the retention of a cocoon-spinning behavior and larval morphological features related to this behavior (Rozen 1984, Michener 2007). Most studies about the biology and nest architecture of the subfamily come from North American (Linsley 1962, Linsley and Cazier 1970, Roberts 1971, Rozen 1984, Rozen and Rozen 1986) and Central American species (Otis et al. 1983, Roubik and Michener 1984, Wuellner and Jang 1996). The knowledge of South American taxa corresponds to brief mentions by Schrottky (1906, 1907) about the biology of *Ptiloglossa ducalis* Smith and *P. matutina* from Brazil and Paraguay and observations of *Cadeguala albopilosa* and *Diphaglossa gayi* from Chile carried out by Claude-Joseph (1926) and Janvier (1933, 1955). Later, Rozen (1984) reinterpreted some aspects of the biology of *Cadeguala* and *Diphaglossa* species. Other studies on *Cadeguala occidentalis* were conducted by Torchio and Burwell (1987) and more recently by Montalva et al. (2011). The biology of some species of the genus *Caupolicana* was studied by Claude-Joseph (1926), Janvier (1933, 1955), Michener (1966), and Genise et al. (1990). The nesting biology of species of *Willinkapis*, *Cadegualina*, *Mydrosoma* and *Mydrosomella* is unknown.



Fossil bee cells with a curved shape attributed to Diphaglossinae have been recently recorded from the Cenozoic of Patagonia, Argentina (Sarzetti et al. 2010). Herein is described the nest architecture and some data on the nesting biology of five South American species of Diphaglossinae, to enable in the future a more accurate comparisons with the fossil examples and to extract paleoenvironmental inferences from them.

In this contribution, novel nesting and biological observations are provided for three Caupolicanini species: *Ptiloglossa matutina* (Schrottkey, 1904), *Ptiloglossa tarsata* (Friese, 1900), and *Zikanapis tucumana* Moure (1945); and two Diphaglossini species: *Cadeguala albopilosa* (Spinola, 1851) and *Diphaglossa gayi* Spinola (1851).

## Materials and methods

Excavations of nests were performed using plastic tubes to trace the tunnels downward while exposing a vertical section of the soil showing the whole structure of the nest when possible. The measures taken were: width and height of the tumulus, maximum diameter and length of the main tunnel and laterals, and number of cells. The length of the vertical part of the cell (from the bottom to the based of the neck), the maximum diameter of the cells, and the diameter of the neck were also measured. The larvae were boiled in water and maintained in alcohol 70%. Samples of soil surrounding nests were collected and carried to the laboratory for studying micromorphology. Collected bees were deposited in the entomological collection of the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN-En), Buenos Aires (Argentina). Dr. Arturo Roig Alsina and Dr. Luis Compagnucci identified the bees. CT images were taken with Elscint SeleCT SP tomograph. 3D-reconstruction of one nest was prepared using computer program SLICER3 v.3.6.3, 2011. SEM images of cell operculum and cocoons were taken with a Philips XL30 SEM at the Museo Argentino de Ciencias Naturales.

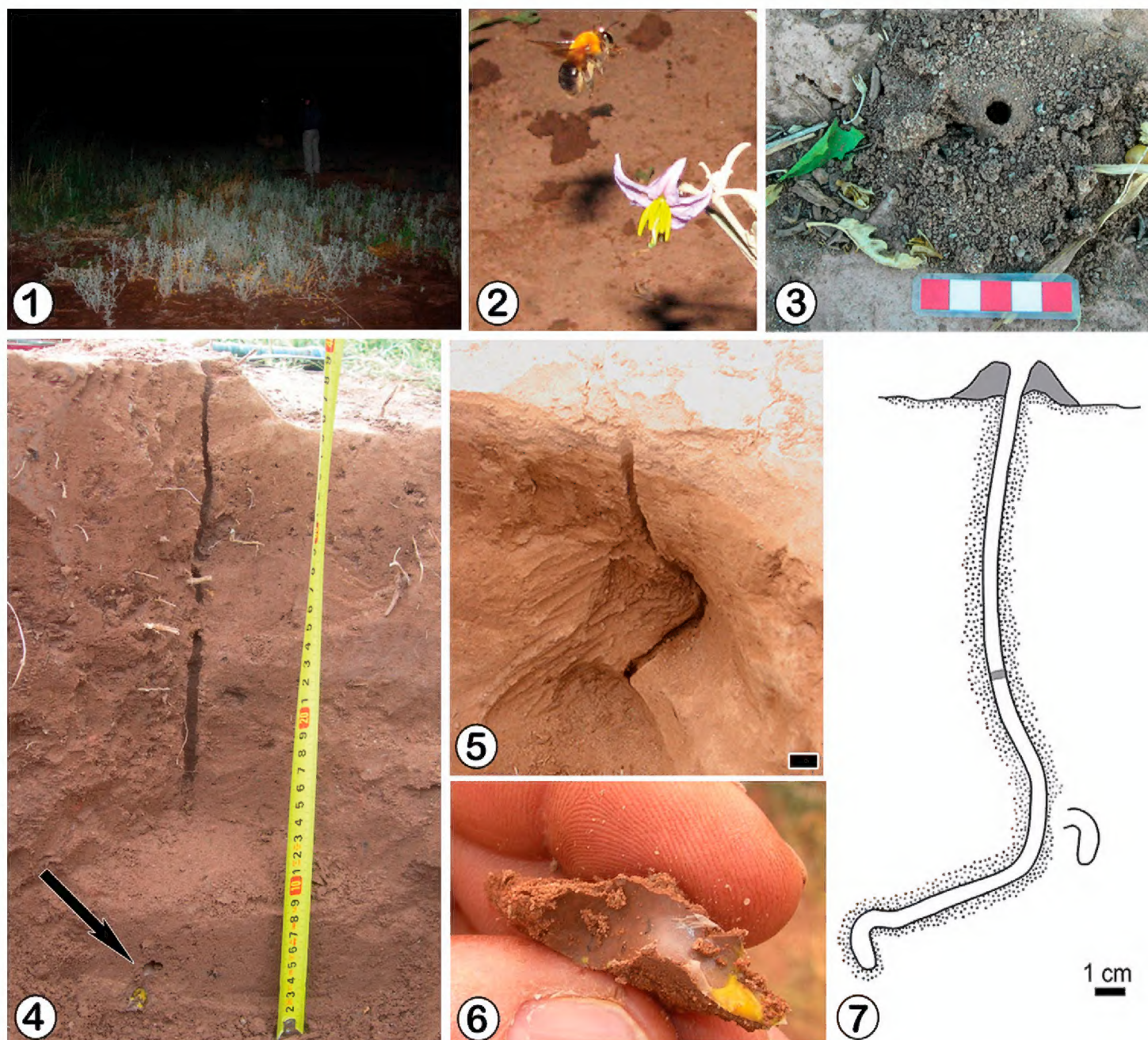
## Results

### *Zikanapis tucumana* (Moure, 1945)

[http://species-id.net/wiki/Zikanapis\\_tucumana](http://species-id.net/wiki/Zikanapis_tucumana)

**Localities and nesting sites.** Observations on the biology and nest structure of this species were carried out during November 24<sup>th</sup>–30<sup>th</sup>, 2008 and through February 3<sup>rd</sup>–5<sup>th</sup>, 2009 at Vinchina (28°49.117'S, 68°11.433'W) and Anillaco (28°48.517'S, 66°55.867'W), both at La Rioja province, northwestern Argentina. The localities are included in xeric environments of the *Larrea*'s shrubland with a mean annual temperature (MAT) around 17° C and a mean annual precipitation (MAP) around 250 mm. Nest entrances were exposed in open areas and were never hidden by stones or shrubs. At Vinchina, nests formed an aggregation at the border of a formerly plowed open area frequently flooded by irrigation (Fig. 1). The ground surface was flat, compacted, and





**Figures 1–7.** *Zikanapis tucumana* (Moure, 1945). **1** General view of the nesting site at Vinchina (La Rioja province) before sunrise **2** Female of *Zikanapis tucumana* during foraging activity **3** Tumulus, turret and open nest entrance **4** Main and lateral tunnel showing one cell at the end (arrow) **5** General view of nest architecture, scale line: 1 cm **6** Remains of a cell with part of the provisions. Note the curvature of neck **7** Nest architecture.

devoid of rocks. The soil was composed principally of fine sand to silt bridged by sparse clayish material. The vegetation was sparse and basically comprising plants of *Solanum* sp. (10 to 20 cm tall) and grasses (Fig. 1). At Anillaco the nests were found at the surroundings of the CRILAR (Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de Anillaco, La Rioja). The ground surface was flat, and the soil consisted of fine sand and numerous rocks of different sizes, which hindered excavation. The vegetation was sparse, consisting of some grasses, and scarce herbaceous plants, such as *Mirabilis ovata* and *Solanum* sp., some cacti, and trees (*Salix* sp. and *Prosopis* sp.). The nests at both localities were located among plants of *Solanum* sp. or near them.

**Daily activity.** *Zikanapis tucumana* was the only species in this study that clearly demonstrated dim-light, matinal foraging. At 04:30 am, still at night, the flower buds of *Solanum* sp. were still closed and no bee activity was observed. Females became



active around 05:00 am, still at night, when the flower buds of *Solanum* sp. began to open (Fig. 2). After 05:20 am, with twilight, females were completely active. With sunrise, around 07:00 am, the foraging activity of the bees ceased almost completely. The activity ended definitively around 07:30 with full daylight, when the entrance of nests were closed from inside with a soil plug. The number of foraging trips per bee during these 150 minutes for the five nests observed was around 8. The females with pollen remained inside nests about 2–3 minutes before leaving again. Their foraging trips lasted about 14 to 17 minutes. During November, males of *Z. tucumana* were also observed flying around nests 1–2 m above ground.

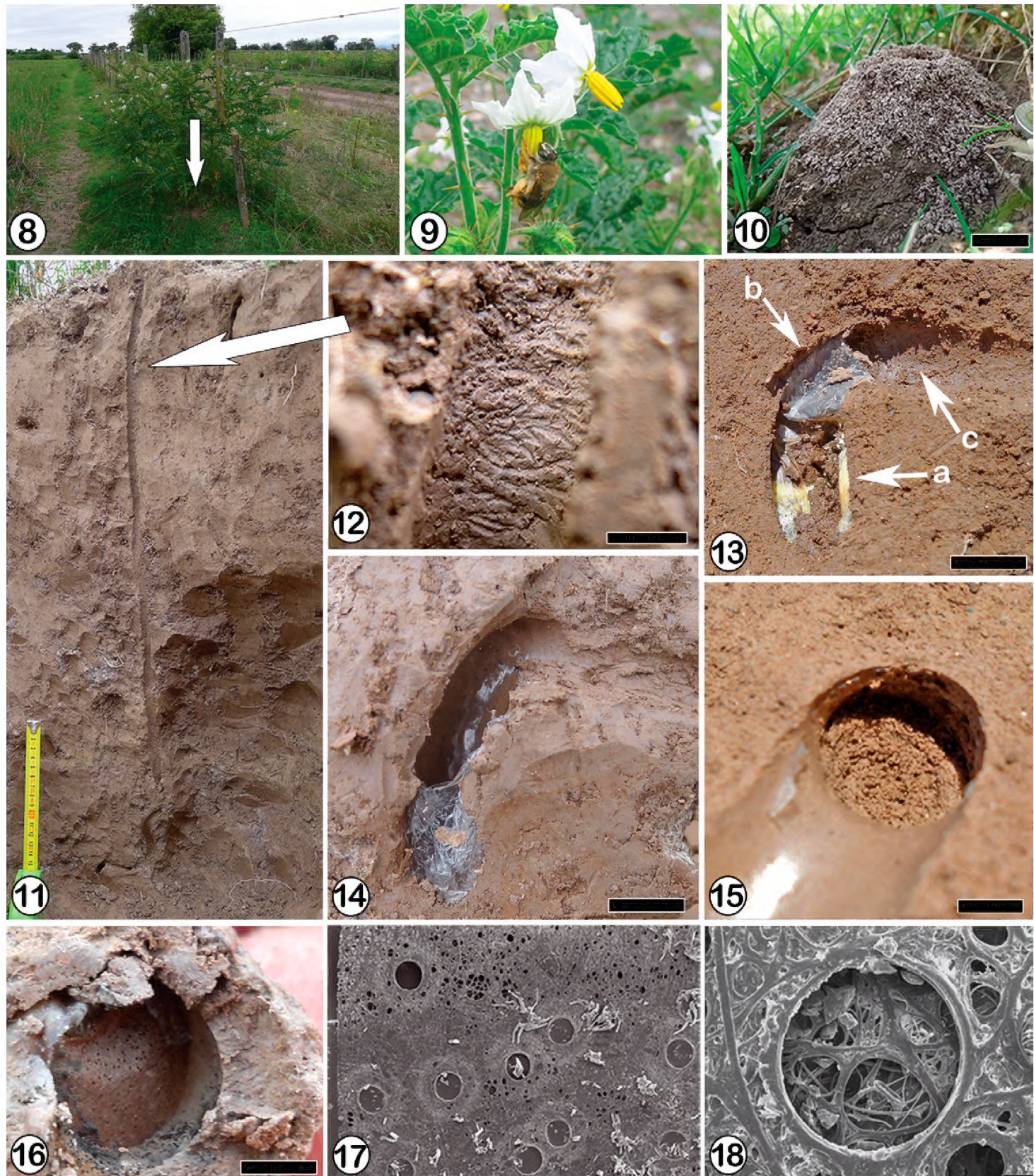
**Description of nests.** The entrance of nests was surrounded by a tumulus that ranged from 5 to 10 cm in diameter and 1 cm high (n: 6). Some nests also had a consolidated turret of 0.8 cm in maximum diameter and 1.7–2 cm high above the entrance (Fig. 3). The entrance, circular and 0.8 cm in diameter, was located at the center of the tumulus. The main tunnel, circular in cross section, was plugged with soil at approximately 10 cm from the entrance when the female was inside the nest. The main tunnel, 16–24 cm long (n: 9), was vertical and mostly straight at Vinchina but sinuous at Anillaco. Three nests had one cell and three other nests two cells. Cells, oriented vertically, were found at depths from 17 to 31 cm. They were disposed radially around the main tunnel, and connected with it by lateral tunnels 6–8 cm long. Lateral tunnels were subhorizontal or slightly inclined downwards and filled with soil when connected with closed cells. They ended in a raised, curved, entrance tunnel connected with the vertical portion of the cell (Figs 4, 5 and 7). Once lined and sealed, the distal part of the entrance tunnel became the curved neck of the cell. The vertical portion of the cells was 1.3–1.8 cm long and 0.9–1 cm in maximum diameter (n: 7). The neck was 0.7–0.8 cm in diameter (n: 2). The inner cell wall, including the neck, was smooth and lined with a whitish, semitransparent, cellophane-like material (Fig. 6). Two cells obtained during November from Vinchina contained eggs. The eggs, whitish, elongate, and slightly curved, were 2.3 mm long and 0.9 mm in maximum diameter. They were laid over the semiliquid provisions. The two cells collected during February at Anillaco contained an egg and a larva respectively. The larva, whitish, immobile and curved was located over a layer of remaining provisions at the base of the cell.

### *Ptiloglossa tarsata* (Friese, 1900)

[http://species-id.net/wiki/Ptiloglossa\\_tarsata](http://species-id.net/wiki/Ptiloglossa_tarsata)

**Locality and nesting site.** The observations on the biology and nest structure were carried out during January 25<sup>th</sup>–30<sup>th</sup>; March 10<sup>th</sup>–16<sup>th</sup>, 2011; and January 4<sup>th</sup>–14<sup>th</sup>, 2012 at Paraje La Florida (25°0.817'S, 65°33.534'W), Salta province, Argentina. Two nests were excavated at this locality, which is in a transitional habitat between rain and dry forest with a MAT around 17 C° and a MAP between 700 to 800 mm (Baudino 1995). Two nests were found in a formerly plowed area in the surroundings of the establishment “La Florida”. The soil surface was flat and the cover was composed mostly





**Figures 8–18.** *Ptiloglossa tarsata* (Friese, 1900). **8** General view of the nesting site at “La Florida” (Salta province), the arrow indicates the location of the nest; **9** Female of *Ptiloglossa tarsata* foraging in a flower of *Solanum* sp. **10** Tumulus of unconsolidated soil, scale line: 1 cm **11** General view showing the nest architecture with a cell at the end of the main tunnel **12** Group of scratches probably produced by female’s mandibles. The arrow indicates their location in the main tunnel; **13** (a) cell with cellophane-like lining and provisions, (b) neck and (c) entrance tunnel, scale line: 1 cm **14** One cell showing the cellophane-like lining on the wall, scale line: 1 cm **15** Spiral closure of one cell, scale line: 0.5 cm **16** Cocoon operculum with holes, scale line: 0.5 cm **17** Scanning electron micrograph of the cocoon operculum showing the fabric of silk threads with small circular holes, scale: 500  $\mu\text{m}$  **18** One circular hole surrounded by silk threads, scale: 50  $\mu\text{m}$ .

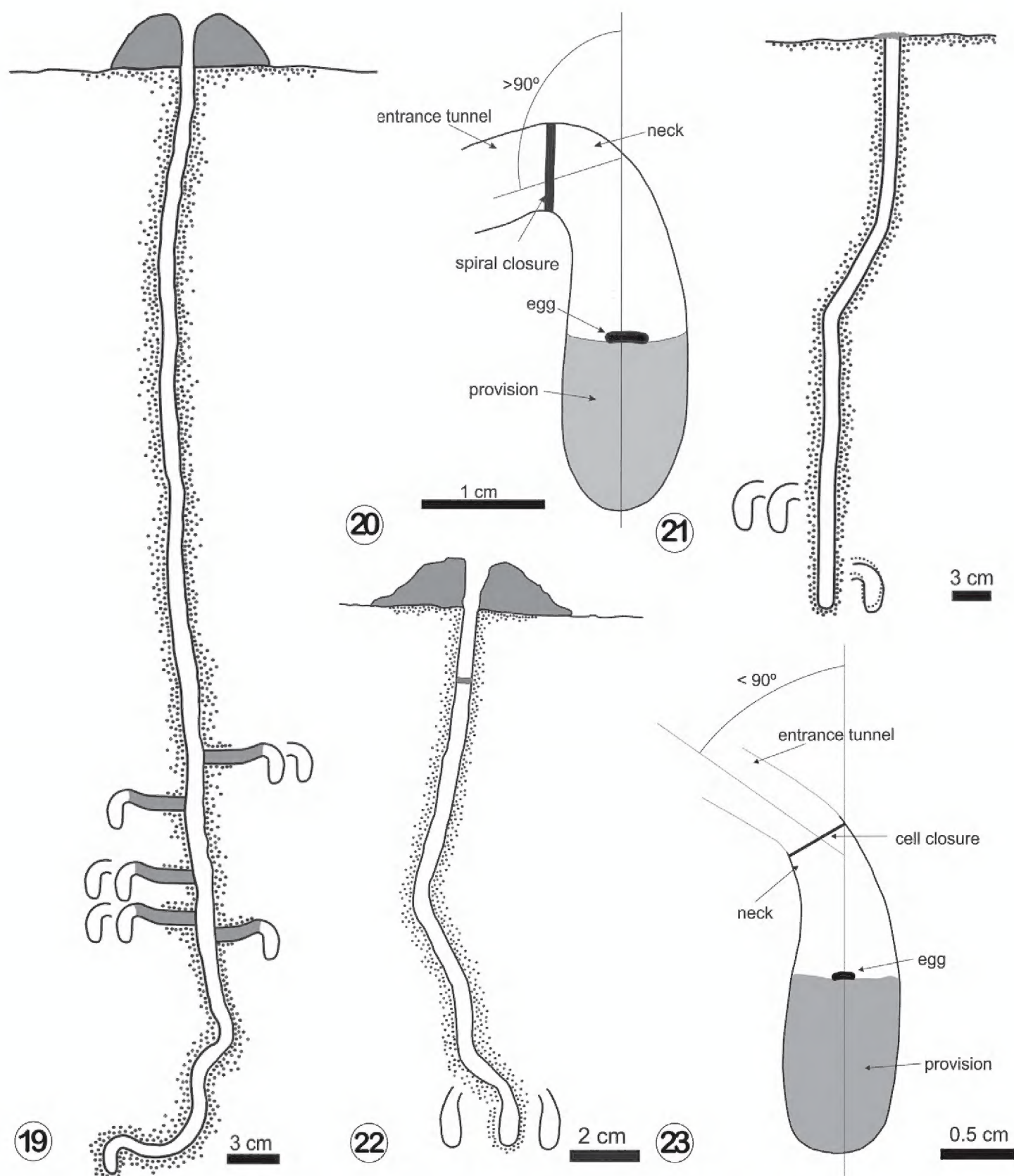


of grasses and plants of *Solanum* sp. and *Clematis* sp. (Fig. 8). The soil consisted of clayish to silty material and was devoid of rocks. The first nest was located next to a fence below a *Solanum* sp. plant, whereas the entrance of the second nest was partially covered by grasses.

**Daily activity.** The foraging activity started about 07:00 am, with full daylight, when females were observed visiting flowers of *Solanum* sp. (Fig. 9). One female made successive foraging trips, remaining inside the nest around 10 minutes after each trip. Foraging trips lasted around 20 minutes. This activity continued until 02:00 pm when the female closed the entrance from inside. The females were inside the main tunnels when the nests were excavated.

**Description of nests.** The entrance of the first nest, circular and 1 cm in diameter, was located at the center of a tumulus, 5.8 cm in maximum diameter and 4 cm high. The tumulus was composed of unconsolidated soil (Fig. 10). The main tunnel, vertical and slightly sinuous, was 40–61 cm long, and 1 cm in maximum diameter (Fig. 11). The inner surface of the main tunnel showed horizontal and densely grouped scratches 1–3 cm long and 0.2 cm wide. These scratches were probably made by the female mandibles (Fig. 12). The cells (n: 12), found at depths of 37–44 cm, were vertical and disposed radially around the main tunnel. Open cells were connected to the main tunnel by horizontal laterals, 5–7 cm long. Laterals ended in a raised, curved, entrance tunnel connecting with the vertical portion of the cell (Figs 11, 13c and 19). Once lined and sealed, the distal part of the entrance tunnel became the curved neck of the cell (Fig. 13b). Each lateral tunnel ended in one or two entrance tunnels connected with cells occurring at the same depth (Fig. 19). Entrance tunnels connected to closed cells were filled with soil (Fig. 19). The vertical portion of a cell was 1.9–2.0 cm long and 0.9–1 cm in maximum diameter (n: 12). The neck was 0.6–0.8 cm in diameter (n: 12). The inner walls of the cells and the neck were smooth and lined with a whitish, semitransparent cellophane-like material that extended up to the closure (Fig. 14). The cell closure, made with soil material, showed a spiral pattern composed of three coils on the inside (Fig. 15). Some cells contained a white wad of cotton-like material. The first nest excavated contained nine cells: three with eggs, three with young larvae, two with mature larvae, and one unfinished empty cell (Fig. 19). The second nest contained four cells, each with an egg. The eggs were whitish, cylindrical and lightly curved, 3 mm long and 0.8 mm wide. They lay on top of the semiliquid provisions (Fig. 20). Young larvae were also whitish, curved, and almost completely submerged in the provisions. Full grown larvae, more yellowish, were found inside cocoons made of a brown, thin, translucent, and slightly coriaceous material. The cocoon operculum is located at the base of the curved neck and consists of a circular disk of translucent, amber, semi-rigid material (Fig. 16). Below the operculum were two more disks of similar structure and composition to it. SEM observations indicated that these three disks were woven structures composed of crossed, coalescent silk threads of different thickness that leave small circular holes of 0.14–0.15 mm in diameter among them (Figs 17 and 18).





**Figures 19–23.** **19** Nest architecture of *Ptiloglossa tarsata* (Fries, 1900). **20** Cell with provisions and egg, cell neck, spiral closure, and entrance tunnel of *Ptiloglossa tarsata* **21** Nest architecture of *Ptiloglossa matutina* (Schrottky, 1904) **22** Nest architecture of *Cadeguala albopilosa* (Spinola, 1851) **23** Cell with provisions and egg, cell neck, location of the cell closure and entrance tunnel of *Cadeguala albopilosa*.

***Ptiloglossa matutina* (Schrottky, 1904)**

[http://species-id.net/wiki/Ptiloglossa\\_matutina](http://species-id.net/wiki/Ptiloglossa_matutina)

**Locality and nesting site.** Observations were carried out during 2012 on March 11<sup>th</sup> and November 10<sup>th</sup> in the Karadya Bioreserve (25°52.233'S, 53°58.167'W), near Andresito, Misiones province, Argentina (Fig. 24). The locality is included in the Upper



Paraná Atlantic Forest Region. The climate is warm subtropical, without a dry season, MAT around 20° C and a MAP around 2000 mm (Servicio Meteorológico Nacional, 2012). During March, 11<sup>th</sup>, males of *Ptiloglossa matutina* were collected, probably before their emergence, inside tunnels exposed in a soil vertical section. On November 10<sup>th</sup>, one nest was excavated in a patch of lateritic soil, altered by human activities, containing abundant roots, litter, and some rocks.

**Daily activity.** The nest was excavated at midday and the female was found inside the main tunnel. Some weeks ago, similar nests were observed with the entrance open and females flying close to them after 06:00 pm (Julián Baigorria, pers. comm.).

**Description of the nest.** The entrance was circular, 0.7 cm in diameter, without tumulus, and closed by a soil plug (Fig. 25). The main tunnel was circular in cross section, 25 cm long, nearly straight and slightly inclined downwards (Figs 21 and 26). It was 0.6–0.7 in maximum diameter. The nest contained four closed cells. One pair at a depth of 20 cm, were possibly connected to the main tunnel by a common tunnel filled with soil, 3 cm long. The other two cells, at a depth of 26 cm, were located at the other side of the main tunnel and also 3–4 cm of it (Fig. 26). The cells were vertical, rounded at the bottom and the neck was strongly curved (Figs 27 and 29). The vertical portion of the cell was 2.3 cm long and 1.3 cm in maximum diameter (n: 4). The neck was 0.8 cm in diameter (n: 4). The inner surface of cells and the neck were lined with a whitish semitransparent, cellophane-like material (Figs 27 and 28). Three cells contained eggs and one a young larva (Figs 27 and 28). The eggs, whitish, cylindrical and lightly curved, were 2.8 mm long and 0.4 mm wide. They laid on top of the yellow semiliquid provisions. The young larva was also whitish, curved, and almost submerged in the provisions. An earthen cell closure was not observed. Instead, a closure of white, cotton-like material that seems to be spirally arranged was observed (Fig. 28).

### *Cadeguala albopilosa* (Spinola, 1851)

[http://species-id.net/wiki/Cadeguala\\_albopilosa](http://species-id.net/wiki/Cadeguala_albopilosa)

**Locality and nesting site.** The observations were carried out during November, 4<sup>th</sup>, 2009 and February, 17<sup>th</sup>, 2011 at Bahia Mansa (43°7.467'S, 71°39.95'W), Parque Nacional Los Alerces, Chubut province, Argentina (Fig. 30). On November, 9<sup>th</sup>, 2009 a nesting site was also found along the route 235 (43°26.142'S, 72°10.017'W), near Yelcho Lake, Palena province (Region de Los Lagos), Chile. The nests were studied at the xeric *Austrocedrus* forest of Bahia Mansa (Parque Nacional Los Alerces) with a MAT around 8 C° and MAP around 1200 mm. The nesting site at Bahia Mansa was slightly sloped and the soil subsurface contained a thin ash layer produced by the Chaiten eruption of May 2008 (Figs 30 and 32). The soil cover was composed of grasses and short herbs (Figs 30 and 31). The nests, around 20 and located among grasses, were distributed in an area of 5 × 7 m. The soil, containing many rocks, roots and grass rhizomes, was composed of silt to very fine sandy material. Nests of sweat bees, aestivation chambers of earthworms, and feeding chambers of cicadas were also present. The Chil-





**Figures 24–29.** *Ptiloglossa matutina* (Schrottky, 1904). **24** General view of the nesting site at Reserva Karadya, Andresito (Misiones province) **25** Nest entrance closed by a plug of soil (arrow), scale line: 1 cm **26** Soil with roots, litter, some rocks, and remains of the main tunnel (arrow) **27** Cell showing the larva partially submerged in provisions, cellophane lining, and the wad cotton-like material attached the cell closure, scale line: 0.5 cm **28** Detail of the cell closure with the cotton-like material **29** Cell and neck wall with the lining removed. Note the high curvature.

ean locality along route 235 was a disturbed forest clearing composed of a dense grass cover. The area had some slopes with large rocks and trunks over the surface. Around 12 nests were distributed in an area of  $6 \times 1.5$  m almost horizontal and partially covered by grasses. At both localities, closest nests were separated each other by 2–4 cm.



**Daily activity.** Bees were observed removing soil from the nests during November, 4th 2009 from 09:00 am to 05:00 pm. In the second field trip, during February 2011, no activity was observed.

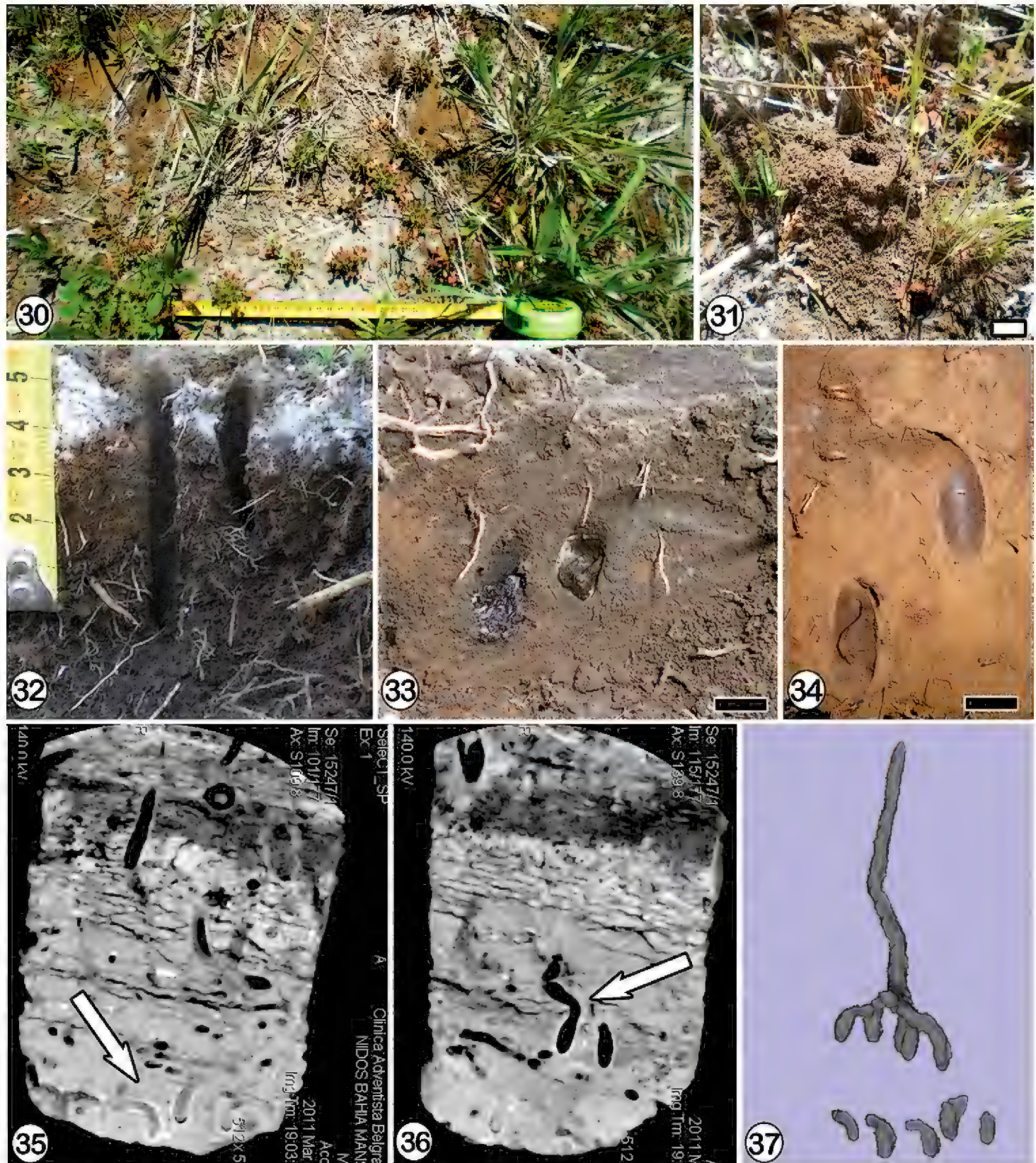
**Description of nests.** Three nests were excavated during November 2009 when bees were active. The entrance, circular, 0.5 cm in maximum diameter was at the center of a large unconsolidated tumulus (Fig. 31). It was composed of a mixture of soil material and ash, 3–3.5 cm in maximum diameter and 2.5 cm high. During February 2011, when no activity of bees was observed, a piece of soil from the nesting site was extracted and taken to the laboratory for examination with a tomograph. The tomographic images provided a more precise picture of the nests and cells (Figs 35–37). The main tunnel, circular in cross section, was plugged with soil 2–3 cm below the entrance and descended vertically, straight or more sinuously among rocks (Fig. 22). It was 18–20 cm long and 0.5 cm in maximum diameter. The cells, found at a depth of 18–20 cm, were vertical and connected to the main tunnel by lateral ones, which were 2.5–3 cm long and mainly horizontal or gently curved (Fig. 23). They were filled with soil when connected to closed cells. At the distal end, lateral tunnels curved downwards, 90° or less, and were connected with the vertical portion of the cell. Once lined and sealed, the more distal curved part of the lateral tunnel connected to the cell became its curved neck (Figs 33 and 34). Each lateral tunnel ended in one or two cells occurring at the same depth (Fig. 22). The number of cells per nest ranged from 2 to 4 in the studied nests (n: 4). The vertical portion of the cells was 1.3–1.6 cm long and 0.6–0.9 cm in maximum diameter (n: 12). The neck was 0.5 cm in diameter (n: 5). The inner walls of the cells and the neck were smooth and lined with a whitish semitransparent cellophane-like material. Cells studied on November contained eggs laying on the surface of the semiliquid provisions. The eggs whitish, cylindrical and slightly curved were 2 mm long and 0.7 mm wide. Cells studied on February contained white pupae inside their cocoons. The cocoons were composed of a brown, translucent, thin, and slightly coriaceous material. The cocoon operculum, located at the base of the curved neck, was composed of one translucent, amber, and semi-rigid circular disk, 5.0–5.5 mm in diameter (n: 2). This disk was a woven structure composed of crossed silk threads of different thickness that leave small circular holes. Below the operculum there was a net of silk threads loosely arranged, similar to the filter-like structure observed by Rozen (1984) in the cocoon of various species of *Diphaglossinae*. Beneath the filter-like structure there was another disk with a similar microstructure to the operculum, but dome-shaped. It was called the ceiling of the pupation chamber by Rozen (1984).

***Diphaglossa gayi* Spinola, 1851**

[http://species-id.net/wiki/Diphaglossa\\_gayi](http://species-id.net/wiki/Diphaglossa_gayi)

**Localities and nesting sites.** The observations were carried out during November 8<sup>th</sup>, 2009 and February 10<sup>th</sup>, 2011 beside the Río Negro Bridge (42°57.433'S, 72°39.233'W) and during November 9<sup>th</sup>, 2009 and February, 15<sup>th</sup>, 2011 at Lonconao (43°13.007'S,





**Figures 30–37.** *Cadeguala albopilosa* (Spinola, 1851). **30** General view of the nest site at Bahia Mansa, Parque Nacional Los Alerces (Chubut province) **31** tumulus of unconsolidated soil **32** two main tunnels and longitudinal view of the soil containing a thin ash layer **33** a pair of cells with provisions, necks and lateral tunnels, scale line: 1 cm **34** remains of cells of other nest, scale line: 1 cm **35–36** tomography images of one block of soil containing *Cadeguala albopilosa* nests, arrows indicate cells **37** 3D-reconstruction of one nest and isolate cells.

71°55.143'W), both localities from the Palena province (Region de Los Lagos, Chile). The nesting sites occur in the glades of hygrophilous evergreen forests with a MAT around 11° C and MAP around 2500–3000 mm. Two nests were excavated. The first nest was located in a steep slope in a farm beside the Río Negro Bridge (Fig. 38), where-





**Figures 38–43.** *Diphaglossa gayi* Spinola, 1851. **38** General view of the nesting site beside the Río Negro Bridge, Chile **39** general view of nesting site at Lonconao, Chile **40** tumulus of soil pellets and open entrance **41** main tunnel **42** nest architecture **43** cell, neck with lining, and egg laying on provisions.

as the second was excavated in a low vertical section of the soil in another farm at Lonconao (Fig. 39). At both localities, the soil, composed of silty to very fine sandy material and devoid of rocks, contained grass rhizomes, some roots, and earthworm burrows. The soil cover consisted of a combination of dense grasses and dicots (Fig. 38), and the subsurface contained a thin ash layer produced by the Chaiten eruption of May 2008.



**Daily activity.** On November 8<sup>th</sup>, 2009 the first nest was found around 06:00 pm and the female was observed entering the nest with pollen around 07:00 pm. On November 9<sup>th</sup>, 2009 the second nest was found also around 06:00 pm and the female was inside the main tunnel.

**Description of nests.** The Rio Negro nest showed an open circular entrance, 0.5 cm in diameter, which was surrounded by an eccentric tumulus, roughly 4.6 cm wide and 5.2 cm long. The tumulus was composed of soil pellets, probably of the recently deposited subsurface ash layer, which were paler than the soil surface (Fig. 40). The Lonconao nest was located in a vertical cut, thus the entrance, 0.7 in diameter, lacked a tumulus. Both nests share the same general structure. The main tunnel, circular in cross section, 32–38 cm long, was nearly straight and slightly inclined downwards, ending in a vertical segment 8 cm long (Figs 41 and 42). Its maximum diameter was 0.7–1.0 cm. Each nest contained four closed cells arranged in two pairs, one pair near the middle portion of the main tunnel, and the other near the end. It is possible that both cells were connected to the main tunnel by a common lateral, filled with soil when the nest was excavated (Fig. 42). The cells were vertical, rounded at the bottom, and the neck was strongly curved (Figs 42 and 43). The vertical portion of the cells was 2 cm long and 1 cm in maximum diameter (n: 8). The neck was 0.7 cm in diameter. The inner surface of cells and the neck was lined with a whitish semitransparent, cellophane-like material. The cells in both nests contained one egg laying on top of the semiliquid provisions. The cell closure was not observed.

## Discussion

The five species studied herein share many ecological preferences, behaviors and features of nest architecture with each other, and with other Diphaglossinae, although some significant differences were also found during this study.

The broad ecological preferences of Diphaglossinae differ greatly among species as shown by its extended geographical distribution (Rozen 1984). Herein are provided values of mean annual temperature (MAT) and mean annual precipitation (MAP), along with vegetation types to understand more precisely this environmental diversity. The southern species, *Diphaglossa gayi* was found in glades of the hygrophilous evergreen forest (MAT = 11° C and MAP = 2500–3000 mm), whereas *Cadeguala albopilosa* nested in the xeric *Austrocedrus* forest (MAT = 8° C and MAP around 1200 mm). The northern species nested besides cultivated fields under warmer conditions (with a MAT between 17° C to 20° C at these localities). *Ptiloglossa tarsata* in an environment that originally corresponded to the more humid (MAP = 700–800 mm) transition between the drier Chaco and the Yungas and *Ptiloglossa matutina* habits the humid Paranaense Atlantic forest (MAP = 2000–2300 mm), while *Zikanapis tucumana* in a more xeric environment (MAP = 250 mm) of the *Larrea* shrubland.

Diphaglossinae were considered traditionally as dim-light bees (Rozen 1984, Michener 2007). However, among the species studied herein, only *Zikanapis tucuma-*



na and possibly *Ptiloglossa matutina* showed dim-light foraging. Wcislo and Tierney (2009) distinguished the following three types of dim-light foraging in bees: (1) matinal, if bees are active before sunrise; (2) vespertine, if bees are active in post-sunset twilight; and (3) crepuscular, if bees are active during both, evening and morning twilight. Females of *Zikanapis tucumana* showed matinal habits, starting the activity even at night (05:00 am), when the flowers of *Solanum* sp. began to open before twilight, which occurred at 05:20 am. Probably, during those 20 minutes there were some twilight, imperceptible for the human eye, which allows bees to fly, and the exposition of the flowers of *Solanum* sp. was synchronized with the start of flights. Females of *Ptiloglossa matutina* were observed flying after 06:00 pm (Julián Baigorria, pers. comm.), which indicates that this species may be vespertine. However, Schrottky (1907) captured *P. matutina* flying at 04:00 am in Paraguay. The observations presented herein indicate that *Ptiloglossa tarsata* is diurnal, starting their activity in the morning and continuing with foraging until 02:00 pm. After 02:00 pm the nests remained open with the female inside. A similar daily activity was recorded for *Cadeguala albopilosa*, which was active from the morning to 07:00 pm. *Diphaglossa gayi* was observed active after 06:00 pm with full sunlight. It was impossible to determine the previous daily activity because nests were found after 06:00 pm.

The proposed advantages of collecting pollen during twilight include: 1) reduction of competition for resources, 2) reduction of predators 3) reduction of nest parasites (e.g. Bohart and Youssef 1976, Roubik 1989, Wcislo et al. 2004, Kelber et al. 2005). In addition, dim-light bees are usually active at cooler temperatures during matinal or vespertine hours and this may reduce the exposure to unfavorable thermal conditions, mainly in those species that lives in xeric habits, montane regions, or higher latitudes (Hurd and Linsley 1970, Kelber et al. 2005, Wcislo and Tierney 2009). Among the three species described herein without dim-light foraging activity, *Diphaglossa gayi* and *Cadeguala albopilosa* inhabits cold-temperate and humid environments, while *Ptiloglossa tarsata* is found under warm and humid conditions. These species are active during the day when competition for food is higher. Accordingly, these species of Diphaglossinae seem to be not significantly affected by the factors mentioned previously. The species with dim-light foraging behavior, *Zikanapis tucumana* and *Ptiloglossa matutina*, inhabits environments as warm as that of *Ptiloglossa tarsata* suggesting that 1) this factor is not responsible for its daily activity, or 2) that *Zikanapis tucumana* and *Ptiloglossa matutina* may be more sensible to temperature than *Ptiloglossa tarsata*, or 3) that the influence of the xeric environment favors the matinal behavior of *Zikanapis tucumana*. Alternatively, the advantage of *Zikanapis tucumana* and *Ptiloglossa matutina* to nest during dim-light hours may be to reduce competitors, predators, or nest parasites.

Some aspects of the nest architecture, as the curvature of entrance tunnels and cell necks, were proposed as advantages to face floodings. For example, Roberts (1971) proposed for *Ptiloglossa guinnae* Roberts nests that the lateral tunnel rose just before the cell neck probably to prevent the entrance of rain water into the cell being provisioned by the female. However, it is difficult to corroborate this hypothesis for all the



species described herein. In nests of *Zikanapis tucumana*, *Ptiloglossa tarsata* and *Ptiloglossa matutina* the end of the lateral tunnels raised and then curved downwards (Figs 7, 19 and 21). The same can be inferred from the strongly curved neck of *Diphaglossa gayi* cells. In contrast, the short lateral tunnels of *Cadeguala albopilosa* nests, curved downwards without raising anteriorly (Fig. 23). Roberts' hypothesis (1971) could be corroborated only for *Diphaglossa gayi*, which nests under MAP around 2500 mm and *Ptiloglossa matutina*, which nests under MAP around 2300 mm, whereas *Cadeguala albopilosa*, which nests under MAP of 1200 mm lacks the raising of the entrance tunnels. In contrast, *Ptiloglossa tarsata* and *Zikanapis tucumana*, which nest under more xeric conditions, 750 mm and 250 mm respectively, show the raised tunnels. Beyond general xeric environmental conditions of the area, *Zikanapis tucumana* nested in a soil frequently flooded by irrigation suggesting that particular conditions of nesting sites may be also involved as a selective agent for this behavior.

There is a tendency of Diphaglossinae species to nest in aggregations of few to many bees (Rozen 1984). These aggregations can persist for more than one generation (Rozen 1984, Torchio and Burwell 1987, Montalva et al. 2011). Among the five species studied here only *Cadeguala albopilosa* and, to a lesser extent, *Zikanapis tucumana* showed aggregations. The nests of *Ptiloglossa tarsata*, *Ptiloglossa matutina*, and *Diphaglossa gayi* were found isolated.

A tumulus, mostly concentric, surrounded the nest entrances in species studied herein that nested in horizontal surfaces as *Zikanapis tucumana*, *Ptiloglossa tarsata*, and *Cadeguala albopilosa*. *Zikanapis tucumana* was the only species that constructed a consolidate tumulus, also observed in *Ptiloglossa arizonensis* Timberlake by Rozen (1984). The nest of *Ptiloglossa matutina* lacked this typical tumulus probably removed by the abundant precipitations. In *Diphaglossa gayi* that nested in sloping surfaces, the tumulus was eccentric or absent in vertical sections of soil. Similar conditions were observed in some species of *Caupolicana* and *Cadeguala* nesting in sloping surfaces (Rozen 1984).

The nest architecture of the species studied here was mostly similar to other Diphaglossinae described in the literature. The main and lateral tunnels were unlined and lack any particular surface texture in all species studied, with the exception of the main tunnel in nests of *Ptiloglossa tarsata*, which showed transverse scratches, probably produced by the female mandibles (Fig. 12).

Depending on the species studied herein, from the main tunnel arose 1 to 9 horizontal lateral tunnels connected with one cell as in *Zikanapis tucumana* or two cells as in *Ptiloglossa tarsata*, *Ptiloglossa matutina*, *Cadeguala albopilosa*, and probably *Diphaglossa gayi*. The presence of two cells connected to the same lateral is a novel feature for Diphaglossinae. In the five species studied here, the lateral tunnels were filled with soil when connected to closed cells. Janvier (1933) mentioned that the lateral tunnels in the nests of *Diphaglossa gayi* were unfilled. However, the closed cells of both nests of *D. gayi* recorded herein were not connected by an open lateral to the main tunnel.

The cell earthen closure of Diphaglossinae nests appears to be similar in all species, as in many bees, showing internally a spiral design (Rozen 1984, Almeida 2008).



This is consistent with the closure found in *Ptiloglossa tarsata* showing three coarse coils (Fig. 15). Similar closures were previously recorded also in *P. arizonensis*, *P. fulvopilosa* Cameron, *Caupolicana gaullei* Vachal, *C. albiventris* Friese, *Cadeguala occidentalis* (Haliday) and *C. albopilosa* (Rozen, 1984). In contrast, Janvier (1933) indicated that *Diphaglossa gayi* apparently lacks an earthen closure, and the same is true for *P. guinnae* Roberts (1971) and *Crawfordapis luctuosa* (Smith) (Rozen 1984). Roberts (1971) suggested that in *P. guinnae* Roberts the lack of a closure could have appeared to facilitate the elimination of CO<sub>2</sub> produced by fermentation of provisions. Similarly in the cells of *Zikanapis tucumana*, *Ptiloglossa matutina*, *Diphaglossa gayi* and *Cadeguala albopilosa* it was impossible to detect an earthen closure. Other character related to the closure was observed in *Ptiloglossa tarsata* and *P. matutina*, whose cells show a wad of cotton-like material of unknown origin (Fig. 28). The same structure was observed by Rozen (1984) in cells of *Ptiloglossa arizonensis* Timberlake. It seems that the presence of this material may be exclusive of *Ptiloglossa* species.

Rozen (1984) indicated that the most outstanding character of all nests of Diphaglossinae is the shape of their cells. These cells are unique among bees in having a curved neck. The shape of diphaglossine brood cells was discussed by Rozen (1984), who described two types of curved cells. Raised tunnels and more curved cells (90° or more degrees of curvature) were found in *Ptiloglossa tarsata*, *P. matutina*, *Zikanapis tucumana*, and *Diphaglossa gayi*, as previously described by Rozen (1984) for other species of *Ptiloglossa* and species of *Crawfordapis*. In contrast, in *Cadeguala albopilosa* (Fig. 23) the lateral tunnel is not raised and accordingly the curvature of cell neck is lesser than 90°, as in species of *Cadeguala* and *Caupolicana* (Rozen 1984). CT images were useful to confirm the curvature of cell necks of *Cadeguala albopilosa* observed in the field (Figs 35–37).

The Diphaglossinae are the only group among the Colletidae whose larvae spin cocoons (Rozen 1984, Michener 2007). Rozen (1984) described in great detail the cocoon structure of several species of the genus *Ptiloglossa* and *Crawfordapis luctuosa* (Smith), and redescribed that of *Cadeguala albopilosa*, previously described by Claude-Joseph (1926). Among the five species studied here, only the cocoons of *Ptiloglossa tarsata* and *Cadeguala albopilosa* were found. The cocoons of both species were similar to those described by Rozen (1984). Their walls were made of a brown, thin, translucent, and slightly coriaceous material. Cells with cocoons are closed by an operculum made of a disk of silk threads, whose fabric is different among species (Rozen 1984). Below the operculum it was described a structure named filter, composed of a net of silk threads that probably enables gas exchange (Roberts 1971, Rozen 1984, Almeida 2008). Immediately beneath the filter it was described another disk, similar in composition and structure to the operculum, but dome-shaped, which is called the ceiling (Rozen 1984). These structures were recognized only in cocoons of *Cadeguala albopilosa*. The observations in one cocoon of *Ptiloglossa tarsata* revealed the presence of one operculum and below it, two other circular disks of similar composition and fabric, which probably had the same function of the filter and the ceiling, but different microstructure.



## Acknowledgements

We thank Arturo Roig Alsina and Luis Compagnucci for the identification of the bees. Daniel Speranza, Martin Umazano, and Ricardo Melchor assisted us during the field trips to Vinchina and Chile. Mirta González identified soil composition, Luis Torino from Paraje La Florida (Salta, Argentina), and Mr. Eliseo from Lonconao (Chile) allowed us to work in their properties. Julián Baigorria assisted us with field observations at the Bioreserva Karadya and Ernesto Krauczuk helped us in Misiones province. Mr. Alaniz, David Gorla and Patricio Fidalgo provided us accommodation in La Rioja. Sebastian Sosa and Jeremias Taborda assisted us with tomography images. We thank all of them. We thank two anonymous reviewers and the Editor for improving the original manuscript. This is a contribution of PICT 1972 from the Agencia Nacional de Promoción Científica y Tecnológica of Argentina to Jorge F. Genise, and PIP 6023 and PIP 80100164 from CONICET to Ricardo Melchor.

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